

Analysis of pre-existing IgG and IgM antibodies against polyethylene glycol (PEG) in the general population

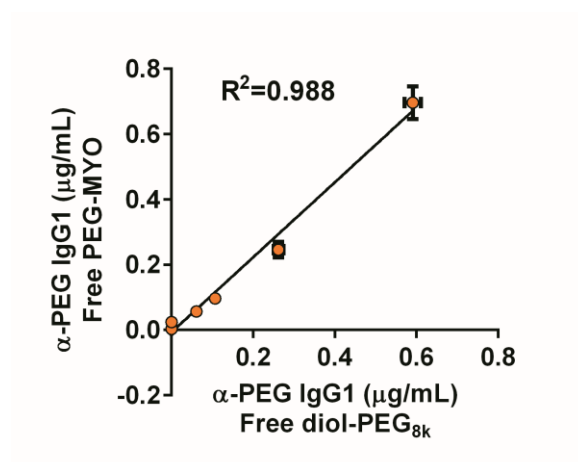
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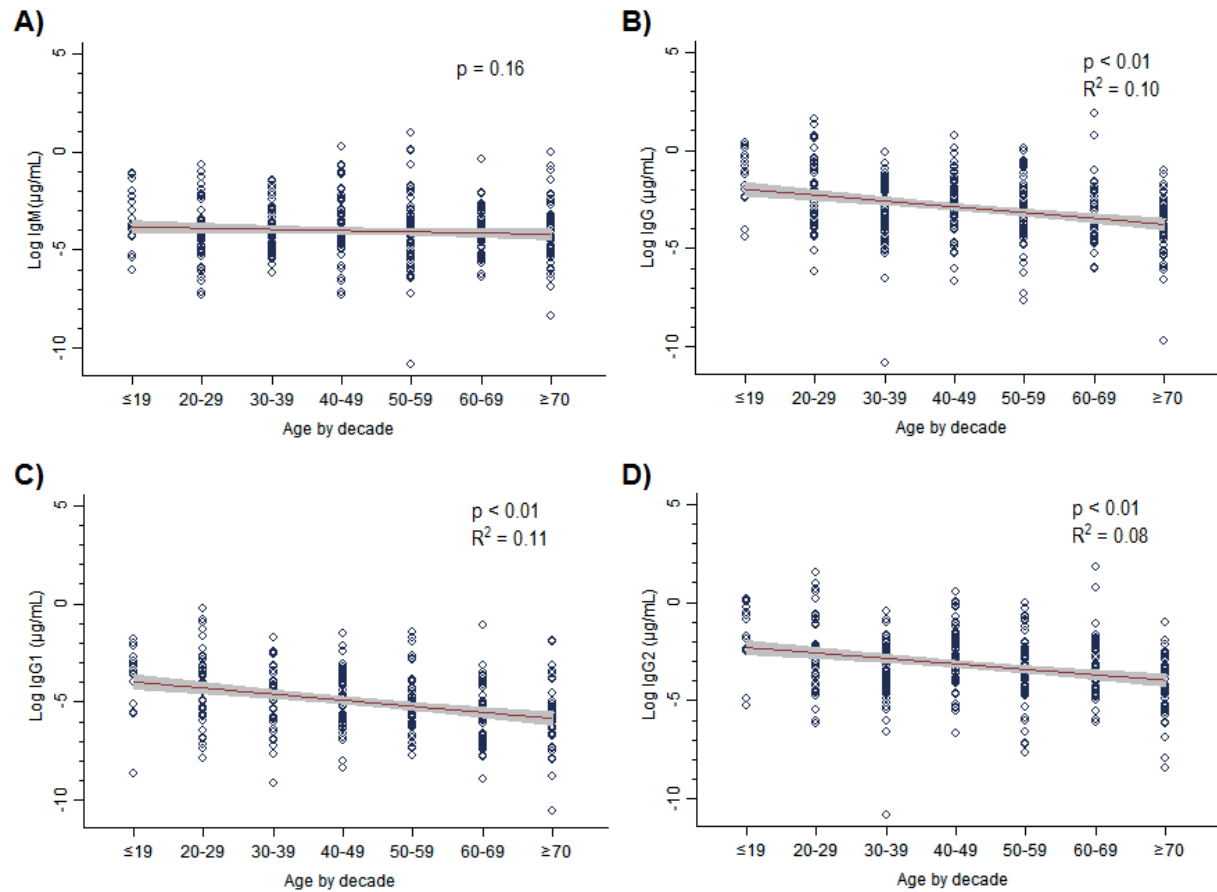
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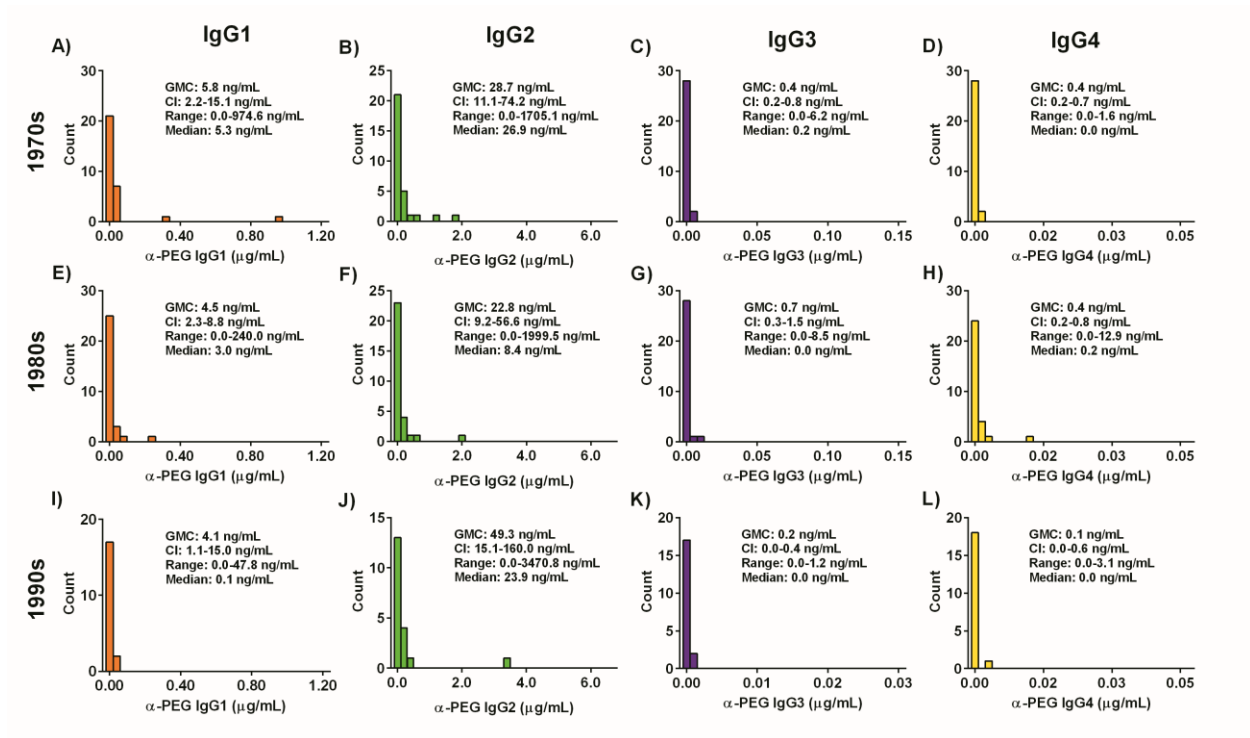
SUPPLEMENTAL FIGURES



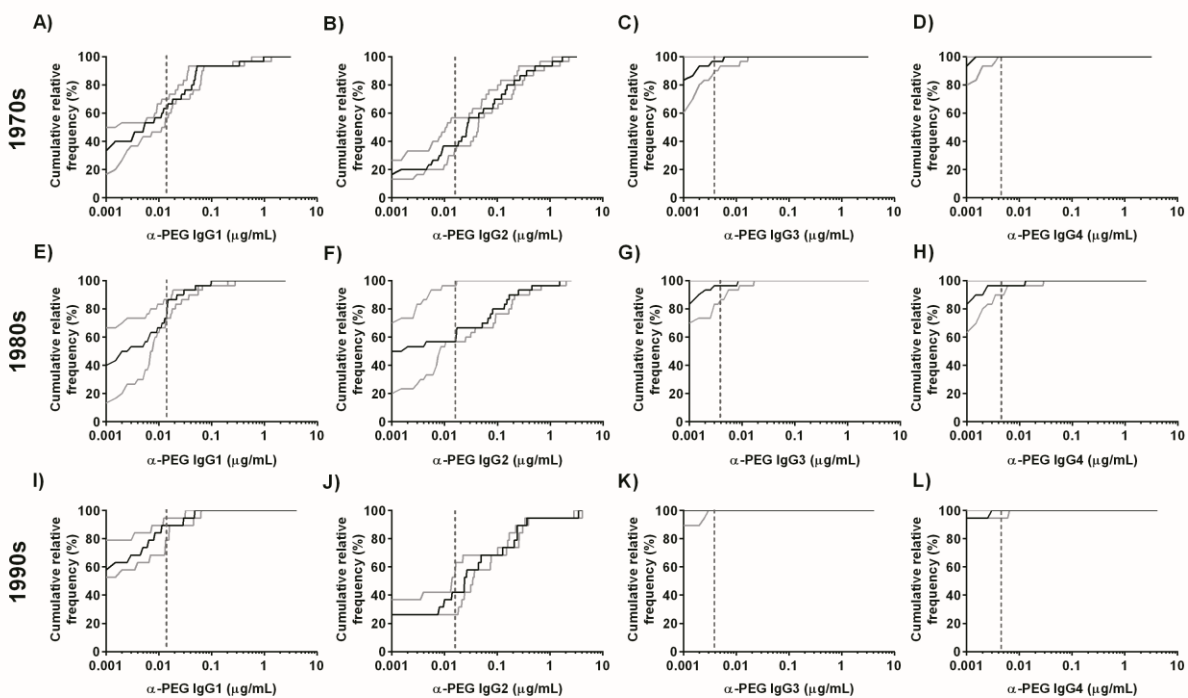
Supplemental Figure 1. Comparison between the anti-PEG IgG levels determined through competitive ELISAs using free diol-PEG_{8k} or free methoxy-PEG_{40k}-myoglobin (PEG-MYO).



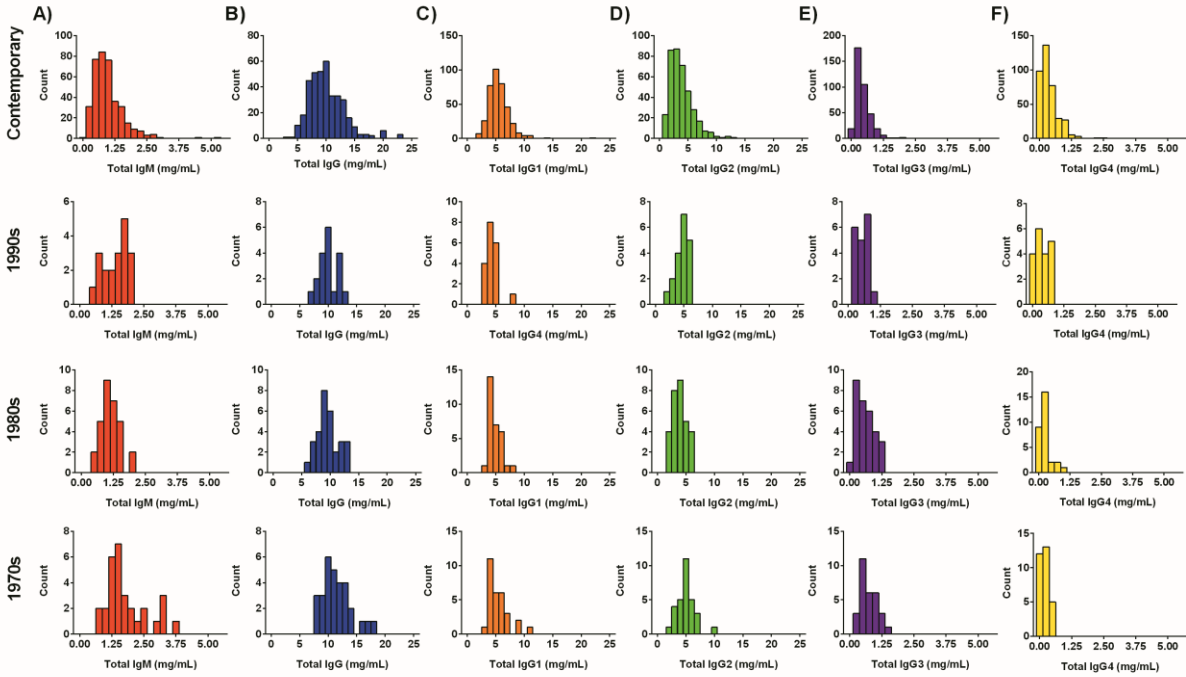
Supplemental Figure 2. Linear regression of log concentrations of A) anti-PEG IgM, B) anti-PEG IgG, C) anti-PEG IgG1, and D) anti-PEG IgG2 concentrations and age by decade. The fitted regression line is shown in red, with 95% confidence intervals shown in gray.



Supplemental Figure 3. Frequency distribution of anti-PEG IgG1 (panels A, E, I), IgG2 (panels B, F, J), IgG3 (panels C, G, K), and IgG4 (panels D, H, L) in human serum samples from the 1970s (panels A-D), 1980s (panels E-H), and 1990s (panels I-L) (n=30, 30, 19, respectively). GMC: geometric mean concentration, CI: 95% confidence intervals for the GMC.



Supplemental Figure 4. Cumulative distribution of anti-PEG IgG1 (panels A, E, I), IgG2 (panels B, F, J), IgG3 (panels C, G, K), and IgG4 (panels D, H, L) in human serum samples from the 1970s (panels A-D), 1980s (panels E-H), and 1990s (panels I-L) (n=30, 30, 19, respectively). Light gray lines represent the 90% CI, and detection cut-off limits are indicated by the vertical gray dashed lines.



Supplemental Figure 5. Frequency distribution of total A) IgM, B) IgG, C) IgG1, D) IgG2, E) IgG3, and F) IgG4 in contemporary human plasma samples (row 1) and serum samples from the 1990s (row 2), 1980s (row 3), and 1970s (row 4) (n=377, 19, 30, 30, respectively).

SUPPLEMENTAL TABLES

Supplemental Table 1. Binding kinetics of chimeric anti-PEG IgG and IgM.

	c6.3 anti-PEG IgG1	c6.3 anti-PEG IgG2	c6.3 anti-PEG IgG3	c6.3 anti-PEG IgG4	cAGP.3 anti-PEG IgM
K_D (M)	$5.8 \pm 0.2 \times 10^{-9}$	$5.3 \pm 0.2 \times 10^{-9}$	$4.8 \pm 0.08 \times 10^{-9}$	$5.1 \pm 0.2 \times 10^{-9}$	$6.6 \pm 0.4 \times 10^{-11}$
k_{on} (1/M•s)	$6.7 \pm 0.07 \times 10^4$	$8.6 \pm 0.1 \times 10^4$	$7.1 \pm 0.04 \times 10^4$	$4.9 \pm 0.04 \times 10^4$	$1.1 \pm 0.03 \times 10^7$
k_{off} (1/s)	$3.9 \pm 0.07 \times 10^{-4}$	$4.6 \pm 0.08 \times 10^{-4}$	$3.4 \pm 0.04 \times 10^{-4}$	$2.5 \pm 0.05 \times 10^{-4}$	$7.1 \pm 0.02 \times 10^{-4}$
R²	0.993	0.993	0.998	0.997	0.974

Supplemental Table 2. ELISA assay details for anti-PEG IgG1, IgG2, IgG3, IgG4, and IgM.

	Anti-PEG IgG1	Anti-PEG IgG2	Anti-PEG IgG3	Anti-PEG IgG4	Anti-PEG IgM
Detection threshold	14.2 ng/mL	15.1 ng/mL	3.9 ng/mL	4.4 ng/mL	6.4 ng/mL
Precision (CV%)	24 ± 6%	24 ± 9%	35 ± 10%	26 ± 6%	32 ± 10%
Accuracy (%)	103 ± 8%	102 ± 4%	99 ± 6%	101 ± 3%	102 ± 4%